# THE EFFECT OF TRYPAN BLUE ON THE GROWTH RATE OF THE CHICK EMBRYO DURING DEVELOPMENT

An abstract of a Thesis by
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The problem. This is a study to determine if the growth rate of the developing chick embryo is changed by the teratogenic agent, trypan blue.

Procedure. The eggs were divided into three groups, unopened controls, saline-injected (0.1 ml, 0.85 NaCl) standards and trypan-blue-injected (0.1 ml, 0.1% solution) experimentals. Injections were made into the yolk sac through the blunt end of the egg during the 48th hour of incubation. Six independent variables, wet weight, dry weight, ash weight, organic content, weight of water and per cent of water of wet weight, were used to determine the effect of the dye on the embryos growth rate. The measurements were taken daily over a 17 day incubation period (4th to 20th day).

Findings. There were no consistently significant differences between the groups but a trend of lower weights for the experimental group developed over the last seven days. Constant death rate and early malformations observed point to the early effect of trypan blue on the chick embryo. On the 14th day significant differences were recorded for five of the six variables between the controls and experimentals.

Conclusions. If there is an effect on total growth of the embryo in later development it is too small to be significant with the sample size used in this study. A long effect on total growth does not appear to be a mechanism for the teratogenicity of trypan blue.

Recommendations. The development around the 14th day might be worth examining further to see if there is a triggering in the metabolic processes which cause the weights to be consistently lower the last seven days of development.

# THE EFFECT OF TRYPAN BLUE ON THE GROWTH RATE OF THE CHICK EMBRYO DURING DEVELOPMENT

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by

Edgar H. Watkins

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# INTRODUCTION AND REVIEW OF THE LITERATURE

Terata have been known and studied for many years. Interest has, until recently, centered on identifying teratogenic agents and listing the various types of defects produced by them. Chemical teratogenic agents which have been tested and identified as causing many malformations include vitamins, hormones, alkylating agents, antibiotics, sulfonamides, agents causing anoxia, azo dyes, heavy metals and many other miscellaneous chemical compounds (Goldstein, Aronow and Kalman, 1968).

Ancel and Lallemand (1941) first reported that trypan blue is a teratogenic agent causing malformation of the caudle bud of the chick embryo. Since that time embryonic malformations have been produced in many species with trypan blue: rat (Gillman et al., 1948; Wilson, Beaudoin and Free, 1959; Beaudoin and Kahlonen, 1963; Beck and Lloyd, 1966; Turbow, 1965), rabbit (Ferm, 1956; Beaudoin and Ferm, 1961), quail (Critchfield and Daniel, 1965), guinea pig (Hoar and Salem, 1961), mouse (Hamburgh, 1954; Barber and Geer, 1964; Waddington and Carter, 1953), and hamster (Ferm, 1957). Ancel (1950) and many others (Beaudoin and Wilson, 1958; Stephan and Sutter, 1961; Kaplan and Grabowski, 1967; and Seichert and Jelinek, 1967) have confirmed to the occurrence of malformations in the chick embryos exposed to trypan blue.

It is evident from these studies that trypan blue has a teratogenic effect on the embryo of many species of animals with the malformations primarily being of the skeleton, neural axis, heart and major blood vessels.

The proposed mechanisms of trypan blue action are numerous. One major group of experimenters believe from their observations that the action of trypan blue is concerned with transport systems and protein content of the plasma. The changes could take place in the mother, causing secondary effects in the embryo, or the changes could take place directly in the embryo. Langman and Van Drunen (1959) reported a significant increase of total protein, albumin and alpha and beta globulins in the maternal rabbit during the first ten days of pregnancy, proposing the alterations in maternal protein could cause the embryonic malformations. Beaudoin and Kahkonen (1963) reported a significant decrease in albumin, beta and alpha-1-globulins concentration and a significant decrease in the weight of trypan-blue-treated fetal rats, proposing teratogenic action may be caused by maternal protein metabolism and the relationship between maternal and fetal protein metabolism. A mesodermatization of the notochord in trypan-blue-treated embryos was attributed to protein aggregation in solution by Waddington and Perry (1956). Lanot (1963) set forth the hypothesis that trypan blue works through RNA-ase, stating that trypan blue cytotoxic action

in the chick embryo does not show characteristic RNA-ase action found in tissue cultures. Lanot (1971), in a cytophotometric study of DNA, shows its synthesis may be inhibited and that the dye works directly on the presomitic mesenchyme by slowing down the formation of the somitic epithelium. An increase in the activity of succinic dehydrogenase in the regions most sensitive to malformations was also recorded. Kaplan and Johnson (1968) observed an increase in oxygen consumption and a decrease in the dry weight of trypan-blue-treated chick embryos. The suggested mechanism was an uncoupling agent which acted like 2,4-dinitrophenol on the tissues of the chick embryo. An accumulation of the dye molecules in the placental or yolk sac tissue which blocks the transport system was given as a possible mechanism by Hamburgh (1954). In the study of fetal rats, Beck, Lloyd and Griffiths (1967) concluded that trypan blue inhibits the lysosomal enzyme action in the intracellular digestion of the visceral yolk sac epithelium. This action would deprive the embryo of important nutrients during critical points in development.

A second group of experimenters believe the malformations occur by increase or decrease in the biological activity of the developing embryo. Vaupel, Nelson and Roux (1961) observed a 30% decrease in the wet weight of treated fetuses suggesting a depression of the growth processes.

Waddington and Carter (1953) stated that the main effect of the dye is general inhibition of development. Ferm (1957) recorded the increase of gross fetal weights of golden hamsters, directly proportional to the amount of trypan blue injected. Stephan and Sutter (1961) proposed a reduction in cellular proliferation of the mesoderm of earlier stages of chick embryos. Lanot (1963, 1971) says there is no mitotic arrest although trypan blue affects the somites and nephrotome. Seichert and Jelinek (1967) stated that there is a growth acceleration with the target sites being regions with intensive growth activity. They also proposed the possibility that the increased growth activity may occur because of previous growth inhibition. Hoar and Salem (1961) reported a 40.3% decrease in weight in 43% of the 30-day-old guinea pig embryos recovered after the mothers were treated with trypan blue. They did not determine if the retardation was physiologically delayed or if it was the early stages of resorption.

The circulatory system and body fluids show a direct effect of trypan blue because of the occurrence of oedemas, blisters and hematomas (Waddington and Carter, 1953; Mulherkar, 1960; Grabowski, 1963; and Kaplan and Grabowski, 1967).

Trypan blue effects the embryo in many ways. The dye can be given under controled conditions and there will be

many changes in the different embryos from minor malformations to major malformations or even death. Many statements have been made about changes in weight and blockage of essential substances during embryonic development which could directly or indirectly influence the growth rate of the embryo. The day-by-day changes in weight and growth rate have not been determined for trypan-blue-treated embryos. Therefore, this study was undertaken to determine the effects of trypan blue on the growth rate by using wet, dry and ash weights, organic content, water content and per cent of water content of the chick embryo from the fourth to the twentieth day of development.

## METHODS AND MATERIALS

Fertile eggs of the white leghorn (Gallus dometicus) were obtained from Hy-Line Poultry Production, Des Moines, Iowa. Seventy-two dozen eggs were divided randomly into three groups and coded to show the age of the embryo when removed. Incubation was at 38°C at a relative humidity of about 56% in a David Bradley electric incubator. The eggs were turned manually at 7:00 AM, 3:00 PM and 10:00 PM. Incubation time was calculated as starting three hours after the eggs were placed in the incubator.

Forty-eight hours from the beginning of incubation the eggs were removed from the incubator. Eggs to be used

as unopened controls were swabbed on the blunt end with 70% alcohol, allowed to dry, then swabbed with Roccal® solution (1:2500 dilution) and set aside while the other two groups were injected.

The standard control group of eggs, after being swabbed as in the control group, was placed in a glass-windowed dust box with two arm openings. The dust box was kept under constant ultraviolet light and washed down with Roccal® solution (1:2500 dilution) before and after each usage. A sterile dissecting needle dipped in 70% alcohol, burned off and allowed to cool was used to open a small hole on the blunt end of each egg. Sterile gloves, tuberculin syringe and a 24 gauge needle were used to inject 0.1 ml of autoclaved sterile 0.85% NaCl saline solution into the yolk sac of the egg. The hole in the egg was sealed with Dupont Duco® cement.

The eggs belonging to the experimental group were handled the same as the standard control group. The saline was replaced with 0.1 ml of 0.1% trypan blue solution made up in saline. The dye solutions were made from powdered stock (Matheson Coleman and Bell; B430 TX1580 July 29, 1960). The dye solution was sterilized with the aid of a millipore filter (pore size 0.4 microns). All three groups were then returned to the incubator at the same time.

Beginning on the fourth day of incubation, a minimum

of ten eggs from each group were removed from the incubator. The viable embryos were dissected out of the egg and washed in saline solution to remove the extra-embryonic membranes and yolk material. Dead embryos and infected eggs were made note of and then discarded. The excess saline solution was removed by blotting with paper toweling. This procedure was carried out daily through the twentieth day of incubation. The embryos were placed in pre-weighed crucibles and weighed on a Mettler balance (type H6 dig. cap. 160g). After weighing, the embryos were placed in drying ovens at 90°C. After a dry weight was obtained the embryos were placed in ashing furnaces (Thermodyne Electric Furnace, F-B1300, Dubuque, Iowa) and ashed at 600°C for two hours. Beginning on the twelfth day a temperature of 600°C and three hours were required to ash the embryos.

Differences between wet and dry weights were interpreted as revealing water content of the embryo. The differences between the dry and ash weight were considered to be the organic content of the embryo. The ash weight was the inorganic content of the embryo.

Records of abnormalities were made by taking pictures of embryos with a Alpa Reflex Camera (Switzerland) on pan-X film (Kodak).

The data was analyzed at the Drake University computer center. The program, PLOTXY, computed the correlation

coefficient and plotted the weights vs. time curve against the regression line. The program RGRSSN computed the multiple regression and analysis of variance for the regression. The Student's t-test was run through the computer to obtain the differences between the three groups. The program STEXTN, computed the growth rates for the six variables.

## DATA

This research was carried out to determine if trypan blue had an effect on the growth rate of the developing chick embryo. The effect of the dye was measured by determining the wet weight, dry weight, ash weight, organic content, weight of water and per cent of water of the wet weight for the period of development from the fourth day to the twentieth day of incubation.

The mean wet weight of the control group increased 29.1208 g with a correlation of 0.87458 between weight and time over a 17-day incubation period. The standards increased 27.9932 g with a correlation of 0.93554 between weight and time and the experimentals increased 27.6485 g with a correlation of 0.87614 between weight and time. The mean wet weight, $\pm$ one standard deviation and the coefficients of variation for each day of incubation are given for each of the three groups in Tables I, VII and XIII. Fig. 1 shows the mean wet weight of the three groups with

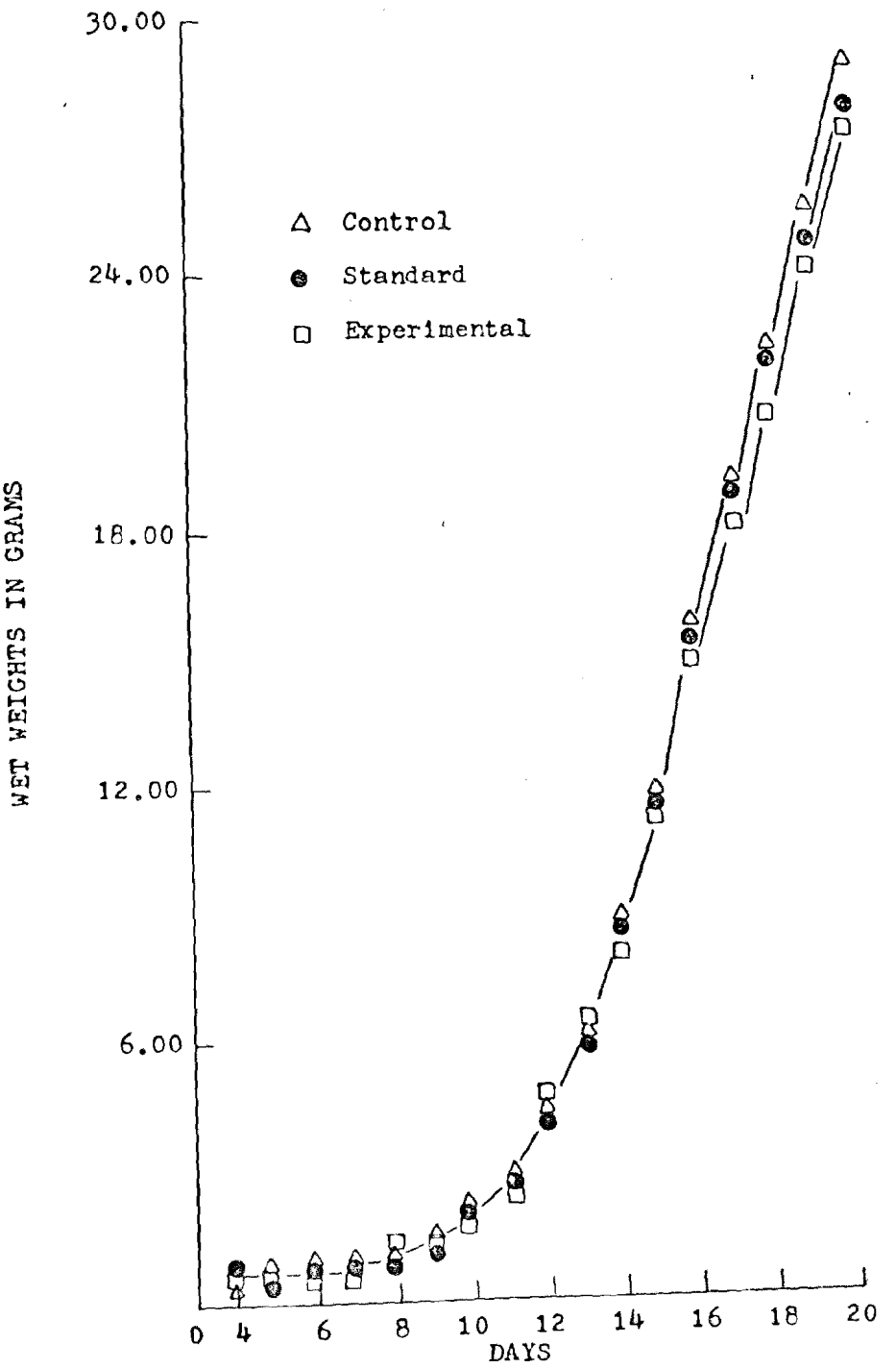


Figure 1. Mean wet weights of control, standard and experimental groups.

respect to time. The mean wet weights of the controls, standards and experimentals appear to be similar for the period of incubation tested. Student's t-test was used to evaluate differences and those that fell outside the 95% confidence interval are marked with an asterisk in the mean tables. Five per cent of the samples tested would be expected to have significant differences by chance.

The mean dry weight of the controls increased 5.6477 g with a correlation of 0.88633 between weight and time over the 17-day incubation period. The standards increased 5.3043 g with a correlation of 0.88427 between weight and time and the experimentals increased 5.1450 g with a correlation of 0.88382 between weight and time. The mean dry weight, $\pm$ one standard deviation and the coefficients of variation for each day of incubation are given for each of the three groups in Tables II, VIII and XIV. Fig. 2 shows the mean dry weight of the three groups with respect to time. The mean dry weights of the controls, standard and experimentals appear to be similar over the period of incubation tested. Student's t-test was used to evaluate differences and those that fell outside the 95% confidence intervals are marked with an asterisk in the mean tables.

The mean ash weight of the controls increased 0.4798 g with a correlation of 0.90442 between weight and time over the 17-day incubation period. The standards increased

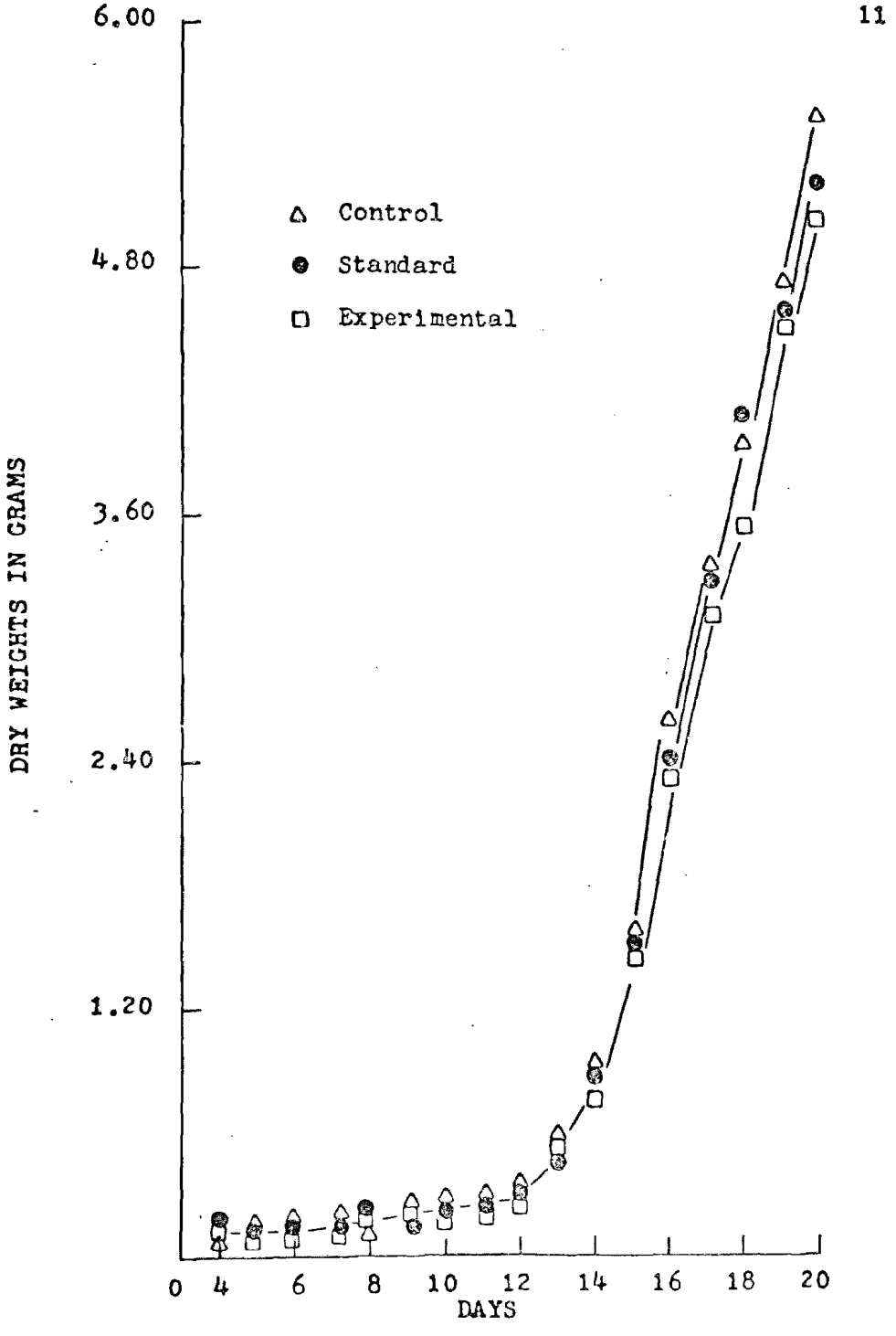


Figure 2. Mean dry weights of control, standard and experimental groups.

0.4687 g with a correlation of 0.90413 between weight and time and the experimentals increased 0.4491 g with a correlation of 0.90933 between weight and time. The mean of the ash weight, ± one standard deviation and the coefficients of variation for each day of incubation are given for each of the three groups in Tables III, IX and XV. Fig. 3 shows the mean ash weight of the three groups with respect to time. Student's t-test was used to evaluate differences and those that fell outside the 95% confidence interval are marked with an asterisk in the mean tables.

The mean organic weight of the controls increased 5.1678 g with a correlation of 0.88445 between weight and time over the 17-day incubation period. The standards increased 4.8357 g with a correlation of 0.88219 between weight and time and the experimentals increased 4.7959 g with a correlation of 0.88109 between weight and time. The mean organic weight, ± one standard deviation and the coefficients of variation for each day of incubation are given for each of the three groups in Tables IV, X and XVI. Fig. 4 shows the mean organic weight with respect to time. The mean organic weights of the controls, standards and experimentals appear to be similar over the period of incubation tested. Student's t-test was used to evaluate differences and those that fell outside the 95% confidence interval are marked with an asterisk in the mean tables.

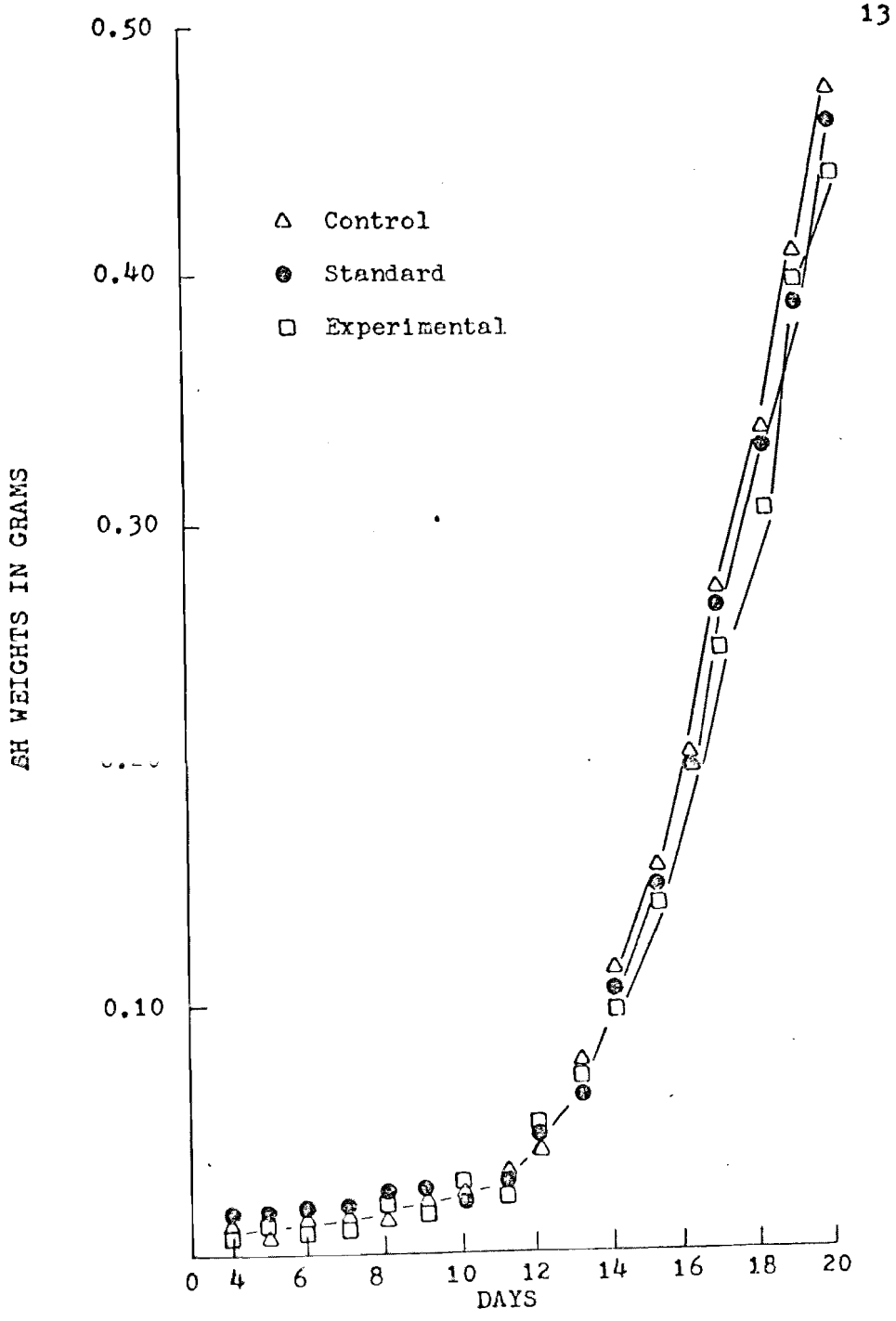


Figure 3. Mean ash weights of control, standard and experimental groups.

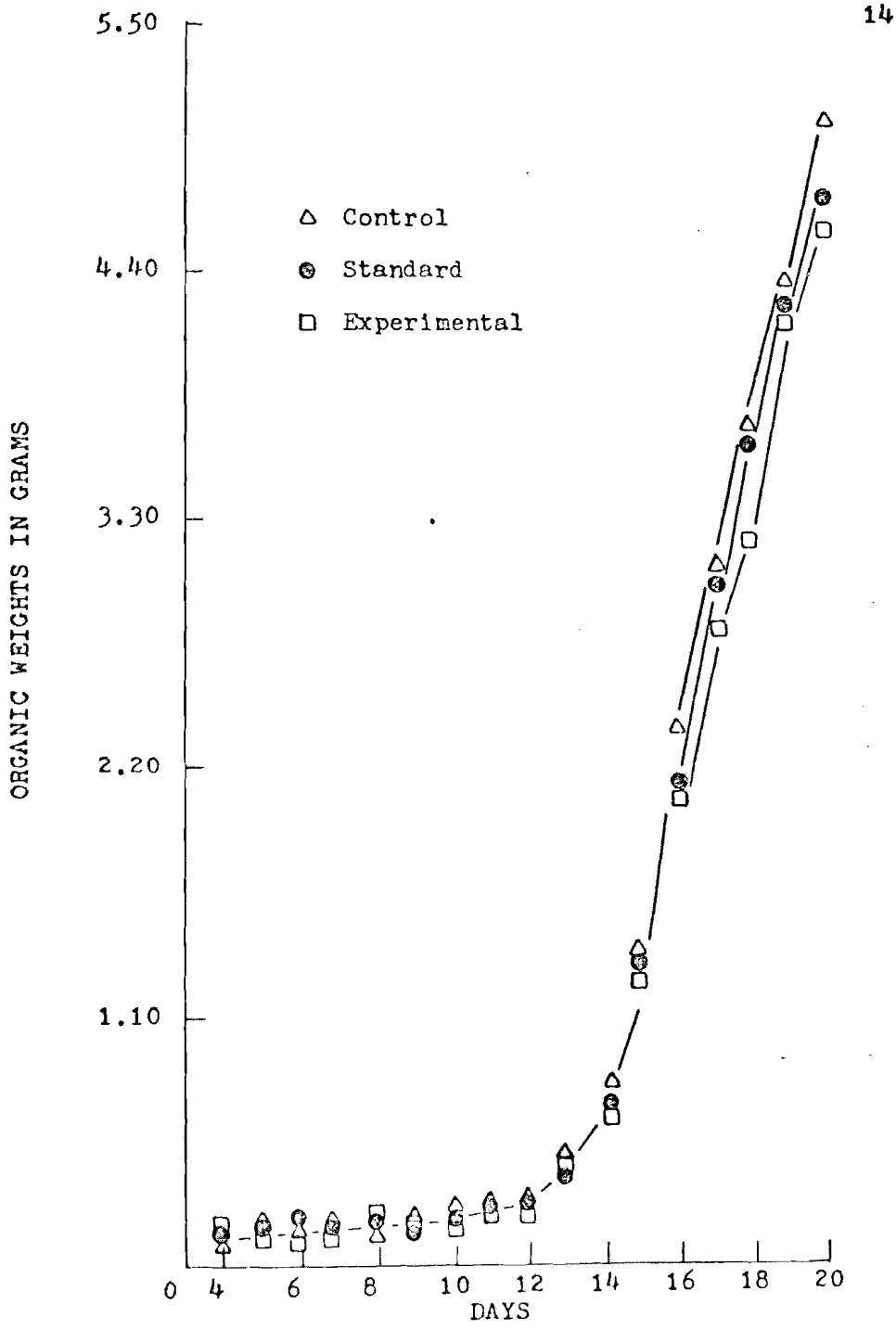


Figure 4. Mean organic weights of control, standard and experimental groups.

The mean weight of water of the controls increased 23.4730 g with a correlation of 0.94443 between weight and time over the 17-day incubation period. The standards increased 22.6889 g with a correlation of 0.94496 between weight and time and the experimentals increased 22.4935 g with a correlation of 0.94545 between weight and time. The mean weight of water, ± one standard deviation and the coefficients of variation for each day of incubation are given in Tables V, XI and XVII. Fig. 5 shows the mean water weights of the three groups with respect to time. The mean weight of water of the controls, standards and experimentals appear to be similar over the period of incubation tested. Student's t-test was used to evaluate differences and those that fell outside the 95% confidence interval are marked with an asterisk in the mean tables.

The mean per cent of water of the controls decreased 13.11% with a correlation of -0.93118 between per cent and time over the 17-day incubation period. The standards decreased 12.57% with a correlation of -0.92741 between per cent and time and the experimentals decreased 11.56% with a correlation of -0.92268 between per cent and time. The mean per cent water, ± one standard deviation and the coefficients of variation for each day of incubation are given in Tables VI, XII and XVIII. Fig. 6 shows the mean per cent water of the three groups with respect to time. The mean

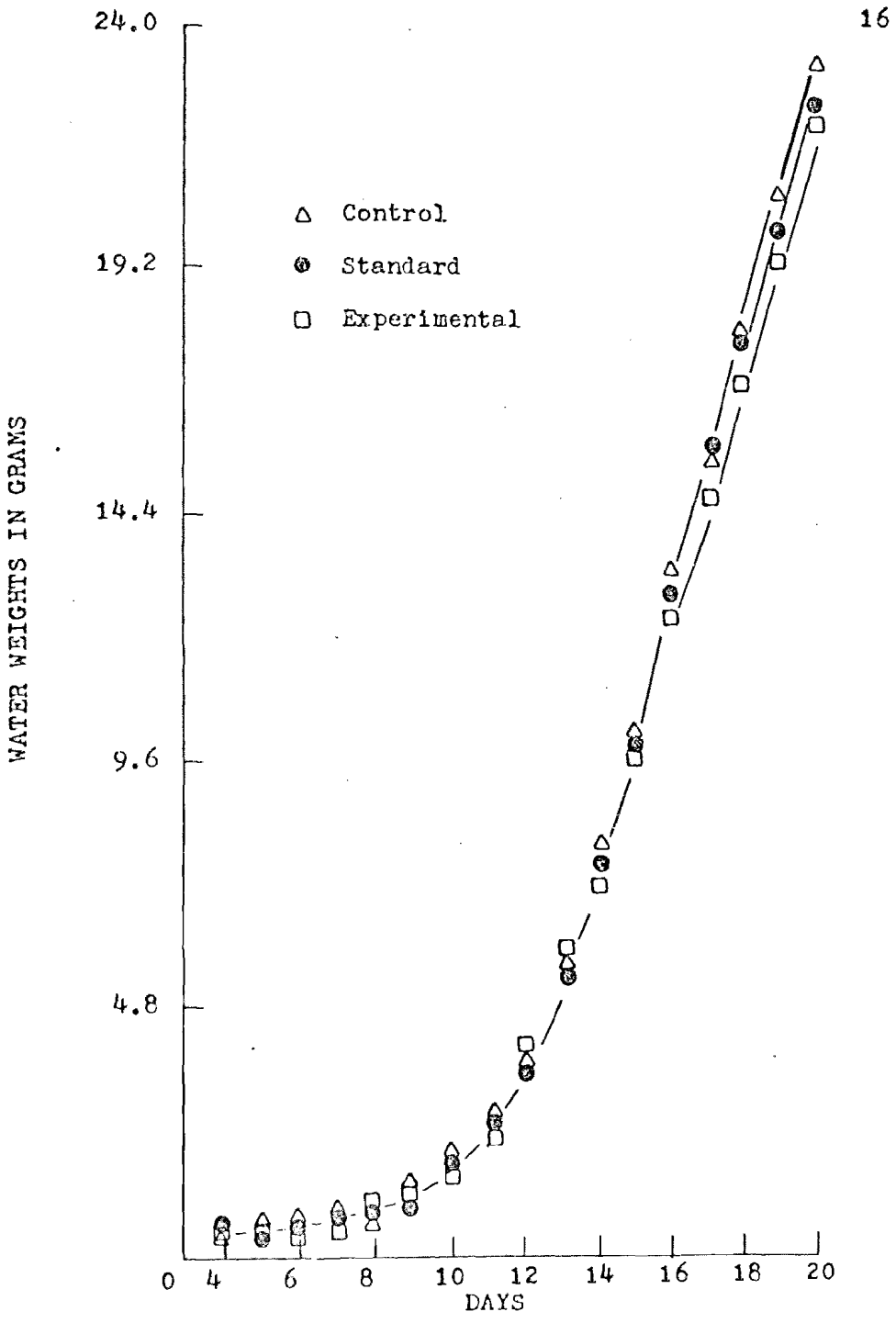


Figure 5. Mean water weights of control, standard and experimental groups.

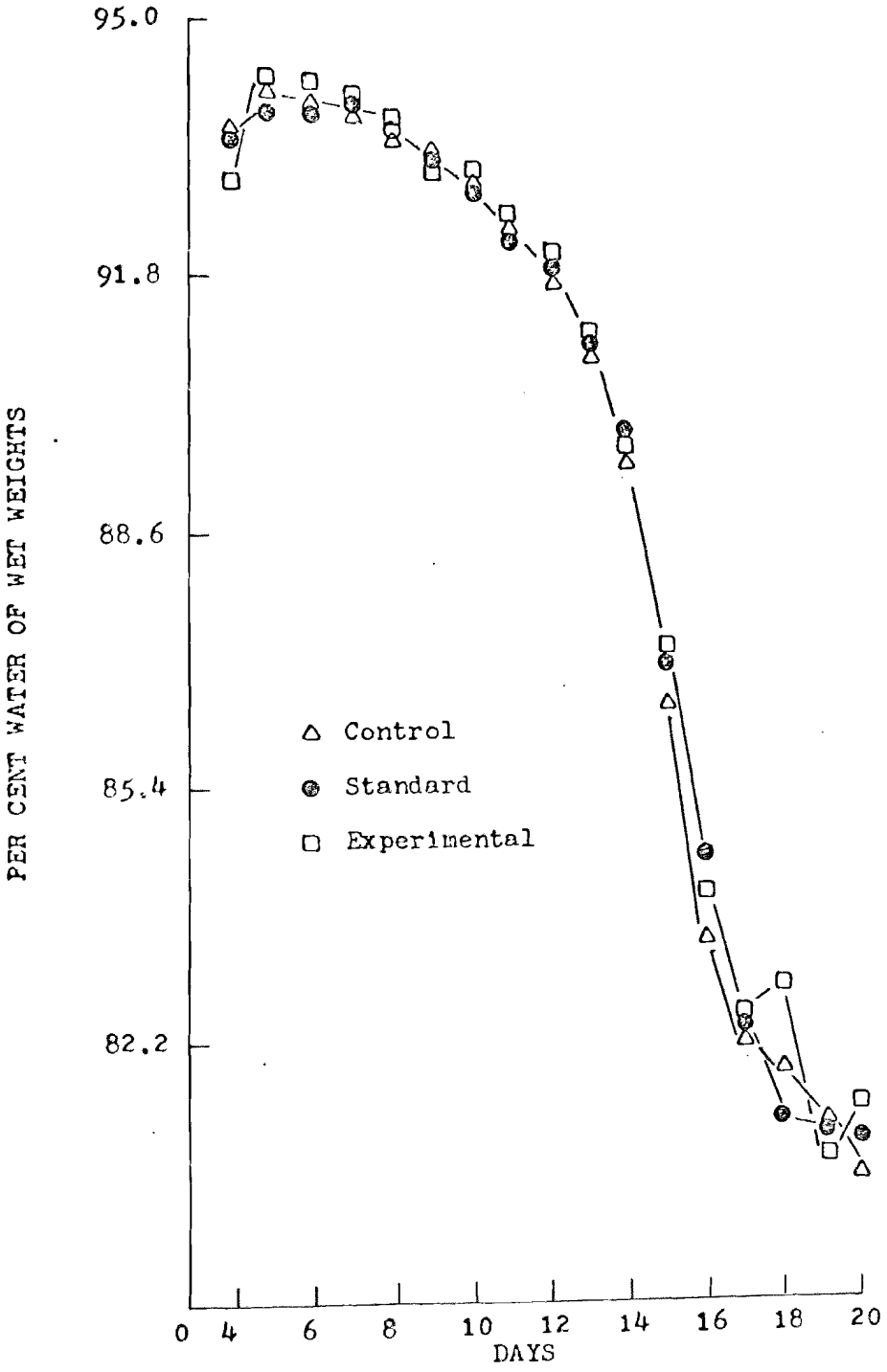


Figure. 6 Mean per cent water of the wet weight of control, standard and experimental groups.

per cent water of the controls, standards and experimentals appear to be similar over the period of incubation tested. Student's t-test was used to evaluate differences and those that fell outside the 95% confidence interval are marked with an asterisk in the mean tables.

The growth rate equation $\log y = \log aa + (bb \log c)x$ was used to determine the change in growth of the six independent variables (Honeywell, 1970). The y-intercepts, slopes and growth rates are listed in Table XIX. The growth rates were very close for each of the six independent variables indicating very little change in the growth rate over the period of incubation (4th day to 20th day).

The mortality rate for the control embryos was 10.6%, the standard 10.3% and 19.5% for the experimental group (chi-square 15.769, $p<.001$). One embryo of the control group was reduced in size and had malformations of the head and body. The only abnormality to appear in the standard embryos was one with a malformed head. The total percentage of abnormalities was 0.55% for the controls and 0.56% for the standards. The first abnormality of the experimentals appeared on the fourth day of incubation and all abnormalities were observed at least once by the seventh day. These same abnormalities continued to appear for the remaining of the incubation period with the exception of the twelfth day when no abnormalities appeared. The total percentage of

abnormalities was 23% for the experimentals (chi-square 351.0, $p<.001$). Abnormalities and the day of their appearance were, head malformations (days 4, 5, 7, 11, 14, 19 and 20), hematoma (5, 7, 9, 11, 14, 16, 17, 18 and 20), body blisters (5, 13, 15, 16 and 20), body and limb malformations (7, 8 and 19) and rumplessness (6, 9, 10 and 20). A 20-day-old embryo is pictured in Figs. 7 and 8. The malformation of the head is very evident with one eye and the upper half of the beak missing. Fig. 9 shows hematoma on the back of the head and neck. Embryos of the control and experimental groups are compared in Fig. 10. The absence of a rump and the reduction in size of the chick in the experimental is apparent.

## DISCUSSION AND CONCLUSIONS

Growth, the increase in weight and mass, is an essential study of the developing embryo. The six variables used in this study give a good picture of growth when taken all together. The growth of the embryo was standardized by two groups for comparison with the trypan-blue-treated embryos.

The increases in wet, dry and water weights of the controls were similar to observations of Romanoff (1967). The standard group corresponded closely to the control group with the groups having only six significant differences. Five significant differences would be expected by chance.

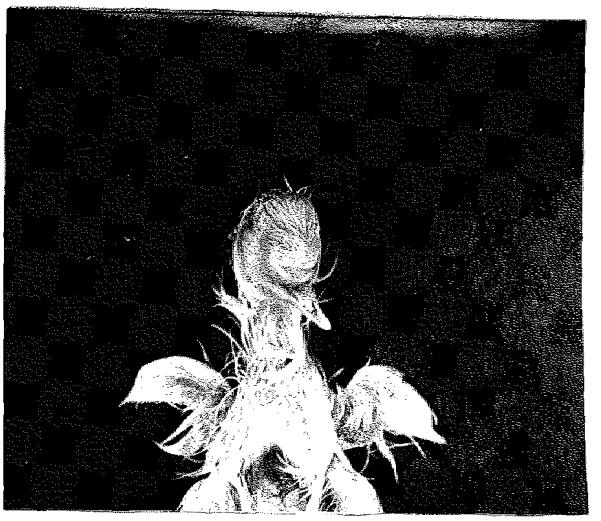

Figure 7. Ventral view of 20-day-old experimental embryo with malformed head.

Figure 8. Lateral view of 20-day-old experimental embryo with malformed head.

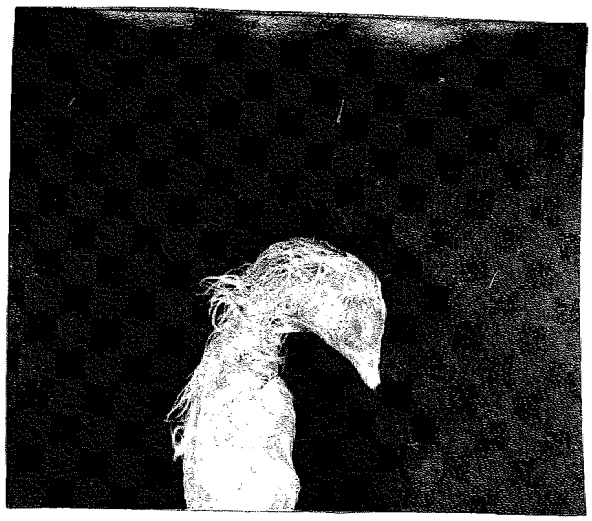

Figure 9. Lateral view of 20-day-old experimental embryo with hematoma on back of head and neck.

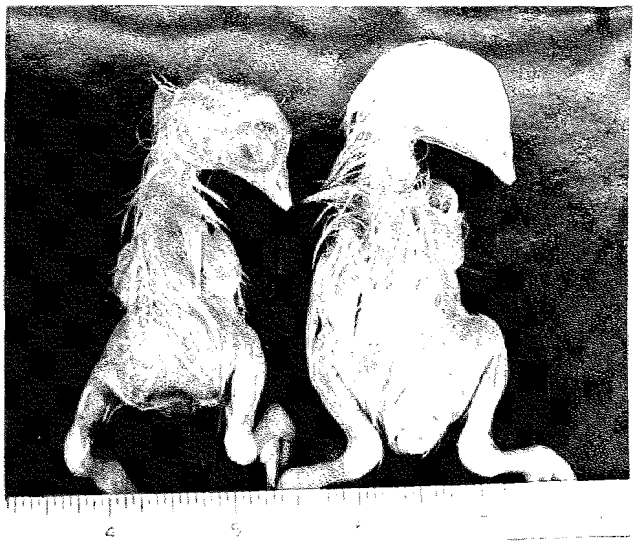

Figure 10. From left to right: experimental and control embryos. Reduction in size and a rumpless condition in the experimental.

The variables with significant differences and the days they occurred were, wet weight (day 9), ash weight (day 8), organic weight (day 9), water weight (day 9) and per cent of water weight (days 16 and 18).

The trypan-blue-treated embryos and the control embryos differed significantly only twelve times. Five significant differences would be expected by chance. The variables with significant differences and the days they occurred were wet weight (days 9 and 14), dry weight (days 4 and 14), ash weight (days 7, 12 and 14), organic weight (days 4 and 14), water weight (days 9 and 14) and per cent of water weight (day 15).

The significant differences between the controls and standards do not seem great enough to set up any patterns or trends. The significant differences between the controls and experimentals do not show a great variation. However, day fourteen where five of the six variables differed significantly was the one possibility of an exception.

It may be necessary to use a larger number of embryos to obtain a statistical difference. Even without the statistical differences between the mean weights there were general trends which appeared in the data. Wet, dry, organic and water weights of the experimentals, without exception, were all lower for the fourteenth through the twentieth days. Ash weights, with the exception of the nineteenth day, were also

consistently lower for these same days. The mean weights for the days prior to the fourteenth day changed randomly as to which group was high or low.

The evidence in this experiment does not agree with the work done by Kaplan and Johnson (1968) with young chick embryos. They found a decrease in dry weight and an increase in oxygen consumption which was significant. If the weight loss or the oxygen consumption were taken individually they would not be significant, however. The time of measurement in their experiment was the sixtieth hour and they used 43 treated embryos. If there is a change in growth during early development it is within the experimental error for the number of embryos used. Seichert and Jelinek (1967) found a significant difference in the extremities but not in the weights of treated embryos. They separated the treated embryos into two groups, the apparently abnormal and the externally normal. The apparently abnormal had a decrease in weight while the externally normal had an increase in weight. In this experiment the abnormal and normal appearing embryos were not separated, thereby possibly off setting one another. However, the abnormal embryos observed here did not appear to be reduced in size unless they were greatly malformed.

The work carried out with mammals, even though the dye is administered through the mother and not directly

into the embryo, appears to be supported by the trends in this experiment. Beaudoin and Kahkonen (1963) and Vaupel et al. (1961) found a decrease in the gross weight of fetal rats. Hoar and Salem (1961) found a decrease in gross weight in their work with guinea pigs. An increase in gross weight of the golden hamster was reported by Ferm (1956). This may be explained by a possible species variation in the effect of the dye.

The abnormalities appeared early in development being observed first on the fourth day of incubation. There were no completely new malformations appearing after the seventh day of development. The number of abnormalities remained fairly constant over the incubation period. Only living embryos were used in determining the mean weight. The experimental embryos that died, generally died early in development. Constant abnormalities and early death of the experimentals would tend to indicate that embryos were malformed early and continued to develop, or died close to the time of treatment.

The one day in the incubation period which might be revealing is the fourteenth day. Significant differences were recorded for five of the six variables and it was the first day of the constantly lower weights for the experimentals. This point in the development of the chick embryo might be worth examining further to see if there is a

triggering in the metabolic processes which cause the weights to be consistently lower.

The early malformations, constant death rates and small differences between the mean weights does not show a long effect on growth rate as a mechanism for teratogenicity of trypan blue. The teratogenic effect seems to take place early in development before any growth trends appear. The similarity in malformations indicates the teratogenic mechanism may be similar in all warm blooded vertebrates. This evidence does not agree with the work of Vaupel et al. (1961), Waddington and Carter (1953), Ferm (1957) or Seichert and Jelinek (1967) on the effects of growth retardation or acceleration. If there is an effect on total growth of the embryo in later development it is too small to be significant with the sample size used in this study.

## SUMMARY

The quantitative effect of trypan blue on the growth rate of white leghorn embryos was determined in this experiment. The eggs were divided into three groups, unopened controls, saline-injcted (0.1 ml, 0.85% NaCl) standards and trypan-blue-injected (0.1 ml, 0.1% solution) experimentals. Six independent variables, wet weight, dry weight, ash weight, organic content, weight of water and per cent of water of wet weight, were used to determine the effect of the dye on the

embryos growth rate. The measurements were taken over a 17-day incubation period (4th to 20th day). The data was analyzed to determine the correlation within the groups and differences between the groups. There were no consistently significant differences between the groups but a trend of lower weights for the experimental group developed over the last seven days of development. Constant death rate and early malformations observed point to the early effect of trypan blue on the chick embryo. A long effect on total growth does not appear to be a mechanism for the teratogenicity of trypan blue.

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# APPENDIX

TABLE I

MEAN WET WEIGHT (GRAMS) CONTROLS

| DAY | N= | MEAN | STD. DEV. | COEF. OF VAR. |
|---|---|---|---|---|
| 4 | 11 | 0.0620 | ±0.0107 | 0.1728 |
| 5 | 14 | 0.2024 | ±0.0477 | 0.2360 |
| 6 | 11 | 0.4543 | ±0.0887 | 0.1952 |
| 7 | 13 | 0.7516 | ±0.0584 | 0.0778 |
| 8 | 10 | 1.1056 | ±0.1241 | 0.1122 |
| 9 | 10 | 1.6477*&*** | ±0.0646 | 0.0392 |
| 10 | 10 | 2.3497 | ±0.0646 | 0.1733 |
| 11 | 10 | 3.2183 | ±0.3263 | 0.1014 |
| 12 | 10 | 4.5599*** | ±0.5523 | 0.1211 |
| 13 | 10 | 6.4469 | ±0.4492 | 0.0743 |
| 14 | 10 | 9.2158 | ±0.7688 | 0.0834 |
| 15 | 10 | 12.0258 | ±1.2725 | 0.1058 |
| 16 | 10 | 16.0986 | ±1.2204 | 0.0758 |
| 17 | 10 | 19.3616 | ±1.2009 | 0.0667 |
| 18 | 10 | 22.4556 | ±3.7374 | 0.1664 |
| 19 | 10 | 25.9392 | ±1.8407 | 0.0710 |
| 20 | 10 | 29.1828 | ±1.5230 | 0.0522 |

* significant difference between control and standard
*** significant difference between control and experimental

TABLE II

MEAN DRY WEIGHT (GRAMS) CONTROLS

| DAY | N= | MEAN | STD. DEV. | COEF. OF VAR. |
|---|---|---|---|---|
| 4 | 11 | 0.0038*** | ±0.0006 | 0.1564 |
| 5 | 14 | 0.0117 | ±0.0039 | 0.3368 |
| 6 | 11 | 0.0273 | ±0.0053 | 0.1959 |
| 7 | 13 | 0.0449 | ±0.0041 | 0.0913 |
| 8 | 10 | 0.0700 | ±0.0081 | 0.1151 |
| 9 | 10 | 0.1088 | ±0.0056 | 0.0519 |
| 10 | 10 | 0.1623 | ±0.0295 | 0.1820 |
| 11 | 10 | 0.2387 | ±0.0254 | 0.1063 |
| 12 | 10 | 0.3660 | ±0.0538 | 0.1470 |
| 13 | 10 | 0.5824 | ±0.0583 | 0.1002 |
| 14 | 10 | 0.9678*** | ±0.1245 | 0.1287 |
| 15 | 10 | 1.6269 | ±0.2671 | 0.1642 |
| 16 | 10 | 2.6472 | ±0.2564 | 0.0968 |
| 17 | 10 | 3.4351 | ±0.3434 | 0.1000 |
| 18 | 10 | 4.0623 | ±0.7381 | 0.1817 |
| 19 | 10 | 4.8445 | ±0.4045 | 0.0835 |
| 20 | 10 | 5.6515 | ±0.3497 | 0.0619 |

*** significant difference between control and experimental

TABLE III

MEAN ASH WEIGHT (GRAMS) CONTROLS

| DAY | N= | MEAN | STD. DEV. | COEF. OF VAR. |
|---|---|---|---|---|
| 4 | 11 | 0.0006 | ±0.0002 | 0.4295 |
| 5 | 14 | 0.0012 | ±0.0006 | 0.5204 |
| 6 | 11 | 0.0034 | ±0.0008 | 0.2189 |
| 7 | 13 | 0.0072*** | ±0.0018 | 0.2446 |
| 8 | 10 | 0.0086* | ±0.0009 | 0.1014 |
| 9 | 10 | 0.0139 | ±0.0011 | 0.0792 |
| 10 | 10 | 0.0210 | ±0.0050 | 0.2367 |
| 11 | 10 | 0.0338 | ±0.0035 | 0.1044 |
| 12 | 10 | 0.0454*** | ±0.0046 | 0.1005 |
| 13 | 10 | 0.0764 | ±0.0075 | 0.0982 |
| 14 | 10 | 0.1135*** | ±0.0118 | 0.1035 |
| 15 | 10 | 0.1584 | ±0.0173 | 0.1093 |
| 16 | 10 | 0.2084 | ±0.0206 | 0.0987 |
| 17 | 10 | 0.2769 | ±0.0153 | 0.0554 |
| 18 | 10 | 0.3445 | ±0.0328 | 0.0953 |
| 19 | 10 | 0.4136 | ±0.0335 | 0.0811 |
| 20 | 10 | 0.4804 | ±0.0220 | 0.0459 |

* significant difference between control and standard
*** significant difference between control and experimental

TABLE IV

MEAN ORGANIC WEIGHT (GRAMS) CONTROLS

| DAY | N= | MEAN | STD. DEV. | COEF. OF VAR. |
|---|---|---|---|---|
| 4 | 11 | 0.0033*** | ±0.0005 | 0.1553 |
| 5 | 14 | 0.0105 | ±0.0038 | 0.3594 |
| 6 | 13 | 0.0239 | ±0.0047 | 0.1971 |
| 7 | 11 | 0.0377 | ±0.0041 | 0.1075 |
| 8 | 10 | 0.0614 | ±0.0075 | 0.1230 |
| 9 | 10 | 0.0950* | ±0.0058 | 0.0606 |
| 10 | 10 | 0.1412 | ±0.0248 | 0.1755 |
| 11 | 10 | 0.2049 | ±0.0223 | 0.1090 |
| 12 | 10 | 0.3206 | ±0.0496 | 0.1547 |
| 13 | 10 | 0.5060 | ±0.0511 | 0.1011 |
| 14 | 10 | 0.8543*** | ±0.1137 | 0.1331 |
| 15 | 10 | 1.4685 | ±0.2505 | 0.1706 |
| 16 | 10 | 2.4387 | ±0.2407 | 0.0987 |
| 17 | 10 | 3.1582 | ±0.3287 | 0.1041 |
| 18 | 10 | 3.7178 | ±0.7086 | 0.1906 |
| 19 | 10 | 4.4309 | ±0.3750 | 0.0846 |
| 20 | 10 | 5.1711 | ±0.3326 | 0.0643 |

* significant difference between control and standard
*** significant difference between control and experimental

TABLE V

MEAN WATER WEIGHT (GRAMS) CONTROLS

| DAY | N= | MEAN | STD. DEV. | COEF OF VAR. |
|---|---|---|---|---|
| 4 | 11 | 0.0582 | ±0.0104 | 0.1787 |
| 5 | 14 | 0.1907 | ±0.0458 | 0.2402 |
| 6 | 11 | 0.4270 | ±0.0834 | 0.1953 |
| 7 | 13 | 0.7067 | ±0.0546 | 0.0772 |
| 8 | 10 | 1.0356 | ±0.1166 | 0.1126 |
| 9 | 10 | 1.5388*&*** | ±0.0596 | 0.0387 |
| 10 | 10 | 2.1874 | ±0.3778 | 0.1727 |
| 11 | 10 | 2.9796 | ±0.3012 | 0.1011 |
| 12 | 10 | 4.1939 | ±0.4999 | 0.1192 |
| 13 | 10 | 5.8645 | ±0.4244 | 0.0724 |
| 14 | 10 | 8.2480*** | ±0.6569 | 0.0796 |
| 15 | 10 | 10.3989 | ±1.0121 | 0.0973 |
| 16 | 10 | 13.4514 | ±0.9750 | 0.0725 |
| 17 | 10 | 15.9265 | ±0.9740 | 0.0612 |
| 18 | 10 | 18.3933 | ±3.0242 | 0.1644 |
| 19 | 10 | 21.0947 | ±1.4950 | 0.0709 |
| 20 | 10 | 23.5312 | ±1.2489 | 0.0531 |

* significant difference between control and standard
*** significant difference between control and experimental

TABLE VI

MEAN PER CENT WATER OF WET WEIGHT CONTROLS

| DAY | N= | MEAN | STD. DEV. | COEF. OF VAR. |
|---|---|---|---|---|
| 4 | 11 | 93.74 | ±1.05 | 0.0112 |
| 5 | 14 | 94.18 | ±1.60 | 0.0170 |
| 6 | 11 | 93.98 | ±0.22 | 0.0023 |
| 7 | 13 | 94.02 | ±0.18 | 0.0019 |
| 8 | 10 | 93.66 | ±0.28 | 0.0029 |
| 9 | 10 | 93.40 | ±0.16 | 0.0017 |
| 10 | 10 | 93.10 | ±0.15 | 0.0016 |
| 11 | 10 | 92.58 | ±0.10 | 0.0011 |
| 12 | 10 | 91.99 | ±0.31 | 0.0034 |
| 13 | 10 | 90.98 | ±0.35 | 0.0038 |
| 14 | 10 | 89.58 | ±0.66 | 0.0074 |
| 15 | 10 | 86.55*** | ±0.89 | 0.0103 |
| 16 | 10 | 83.58* | ±0.51 | 0.0061 |
| 17 | 10 | 82.28 | ±0.72 | 0.0087 |
| 18 | 10 | 81.96* | ±0.84 | 0.0102 |
| 19 | 10 | 81.33 | ±0.79 | 0.0097 |
| 20 | 10 | 80.63 | ±0.66 | 0.0082 |

* significant difference between control and standard
*** significant difference between control and experimental

TABLE VII

MEAN WET WEIGHT (GRAMS) STANDARDS

| DAY | N= | MEAN | STD. DEV. | COEF. OF VAR. |
|---|---|---|---|---|
| 4 | 11 | 0.0766 | ±0.0252 | 0.3287 |
| 5 | 11 | 0.1875 | ±0.0316 | 0.1683 |
| 6 | 12 | 0.4509 | ±0.0659 | 0.1462 |
| 7 | 10 | 0.7505 | ±0.0840 | 0.1120 |
| 8 | 10 | 1.1713 | ±0.1091 | 0.0931 |
| 9 | 10 | 1.5631* | ±0.0887 | 0.0568 |
| 10 | 10 | 2.2324 | ±0.0784 | 0.0351 |
| 11 | 10 | 3.1636 | ±0.2365 | 0.0748 |
| 12 | 10 | 4.5540 | ±0.5482 | 0.1204 |
| 13 | 10 | 6.2391 | ±0.5617 | 0.0900 |
| 14 | 10 | 8.7784 | ±0.7056 | 0.0804 |
| 15 | 10 | 11.8119 | ±1.0941 | 0.0926 |
| 16 | 10 | 15.5675 | ±1.9419 | 0.1247 |
| 17 | 10 | 19.3087 | ±1.6698 | 0.0865 |
| 18 | 10 | 22.3754 | ±2.6332 | 0.1177 |
| 19 | 10 | 24.9714 | ±1.1328 | 0.0454 |
| 20 | 10 | 28.0698 | ±1.9412 | 0.0692 |

* significant difference between control and standard

TABLE VIII

MEAN DRY WEIGHT (GRAMS) STANDARDS

| DAY | N= | MEAN | STD. DEV. | COEF OF VAR. |
|---|---|---|---|---|
| 4 | 11 | 0.0048 | ±0.0015 | 0.3062 |
| 5 | 11 | 0.0113 | ±0.0017 | 0.1504 |
| 6 | 12 | 0.0273 | ±0.0052 | 0.1903 |
| 7 | 10 | 0.0447 | ±0.0056 | 0.1243 |
| 8 | 10 | 0.0741 | ±0.0090 | 0.1220 |
| 9 | 10 | 0.1037 | ±0.0063 | 0.0609 |
| 10 | 10 | 0.1559 | ±0.0069 | 0.0445 |
| 11 | 10 | 0.2374 | ±0.0183 | 0.0771 |
| 12 | 10 | 0.3639 | ±0.0472 | 0.1297 |
| 13 | 10 | 0.5547 | ±0.0709 | 0.1277 |
| 14 | 10 | 0.8892 | ±0.0820 | 0.0922 |
| 15 | 10 | 1.5317 | ±0.2202 | 0.1437 |
| 16 | 10 | 2.4039 | ±0.4685 | 0.1949 |
| 17 | 10 | 3.3744 | ±0.3734 | 0.1107 |
| 18 | 10 | 4.1626 | ±0.4721 | 0.1134 |
| 19 | 10 | 4.6904 | ±0.3325 | 0.0709 |
| 20 | 10 | 5.3091 | ±0.6561 | 0.1236 |

TABLE IX

MEAN ASH WEIGHT (GRAMS) STANDARDS

| DAY | N= | MEAN | STD. DEV. | COEF. OF VAR. |
|---|---|---|---|---|
| 4 | 11 | 0.0007 | ±0.0003 | 0.3768 |
| 5 | 11 | 0.0015 | ±0.0005 | 0.3632 |
| 6 | 12 | 0.0036 | ±0.0006 | 0.1764 |
| 7 | 10 | 0.0075** | ±0.0016 | 0.2165 |
| 8 | 10 | 0.0106* | ±0.0018 | 0.1673 |
| 9 | 10 | 0.0143 | ±0.0012 | 0.0815 |
| 10 | 10 | 0.0209 | ±0.0015 | 0.0697 |
| 11 | 10 | 0.0325 | ±0.0030 | 0.0931 |
| 12 | 10 | 0.0500 | ±0.0068 | 0.1365 |
| 13 | 10 | 0.0691 | ±0.0095 | 0.1374 |
| 14 | 10 | 0.1082 | ±0.0107 | 0.0987 |
| 15 | 10 | 0.1514 | ±0.0225 | 0.1489 |
| 16 | 10 | 0.2052 | ±0.0208 | 0.1012 |
| 17 | 10 | 0.2744 | ±0.0323 | 0.1178 |
| 18 | 10 | 0.3382 | ±0.0376 | 0.1111 |
| 19 | 10 | 0.3933 | ±0.0195 | 0.0495 |
| 20 | 10 | 0.4694 | ±0.0418 | 0.0891 |

* significant difference between control and standard
** significant difference between standard and experimental

TABLE X

MEAN ORGANIC WEIGHT (GRAMS) STANDARDS

| DAY | N= | MEAN | STD. DEV. | COEF. OF VAR. |
|---|---|---|---|---|
| 4 | 11 | 0.0041 | ±0.0013 | 0.3220 |
| 5 | 11 | 0.0099 | ±0.0015 | 0.1534 |
| 6 | 12 | 0.0238 | ±0.0052 | 0.2180 |
| 7 | 10 | 0.0372 | ±0.0054 | 0.1455 |
| 8 | 10 | 0.0634 | ±0.0075 | 0.1180 |
| 9 | 10 | 0.0893* | ±0.0056 | 0.0630 |
| 10 | 10 | 0.1349** | ±0.0064 | 0.0474 |
| 11 | 10 | 0.2049 | ±0.0157 | 0.0765 |
| 12 | 10 | 0.3139 | ±0.0411 | 0.1310 |
| 13 | 10 | 0.4855 | ±0.0639 | 0.1316 |
| 14 | 10 | 0.7810 | ±0.0717 | 0.0918 |
| 15 | 10 | 1.3804 | ±0.2155 | 0.1561 |
| 16 | 10 | 2.1987 | ±0.4526 | 0.2059 |
| 17 | 10 | 3.1000 | ±0.3425 | 0.1105 |
| 18 | 10 | 3.8244 | ±0.4353 | 0.1138 |
| 19 | 10 | 4.2971 | ±0.3185 | 0.0741 |
| 20 | 10 | 4.8398 | ±0.6230 | 0.1138 |

* significant difference between control and standard
** significant difference between standard and experimental

TABLE XI

MEAN WATER WEIGHT (GRAMS) STANDARDS

| DAY | N= | MEAN | STD. DEV. | COEF. OF VAR. |
|---|---|---|---|---|
| 4 | 11 | 0.0718 | ±0.0238 | 0.3318 |
| 5 | 11 | 0.1762 | ±0.0300 | 0.1700 |
| 6 | 12 | 0.4236 | ±0.0611 | 0.1457 |
| 7 | 10 | 0.7058 | ±0.0787 | 0.1115 |
| 8 | 10 | 1.0972 | ±0.1012 | 0.0923 |
| 9 | 10 | 1.4594* | ±0.0832 | 0.0570 |
| 10 | 10 | 2.0765 | ±0.0719 | 0.0349 |
| 11 | 10 | 2.9262 | ±0.2191 | 0.0749 |
| 12 | 10 | 4.1901 | ±0.5014 | 0.1197 |
| 13 | 10 | 5.6844 | ±0.4942 | 0.0869 |
| 14 | 10 | 7.8892 | ±0.6254 | 0.0793 |
| 15 | 10 | 10.2802 | ±0.8857 | 0.0862 |
| 16 | 10 | 13.1637 | ±1.4961 | 0.1137 |
| 17 | 10 | 15.9343 | ±1.3342 | 0.0837 |
| 18 | 10 | 18.2128 | ±2.1667 | 0.1190 |
| 19 | 10 | 20.2810 | ±0.9112 | 0.0449 |
| 20 | 10 | 22.7607 | ±1.4119 | 0.0620 |

* significant difference between control and standard

TABLE XII

MEAN PER CENT WATER OF WET WEIGHT STANDARDS

| DAY | N= | MEAN | STD. DEV. | COEF. OF VAR. |
|---|---|---|---|---|
| 4 | 11 | 93.70 | ±0.93 | 0.0099 |
| 5 | 11 | 93.94** | ±0.29 | 0.0030 |
| 6 | 12 | 93.94 | ±0.59 | 0.0062 |
| 7 | 10 | 94.05 | ±0.22 | 0.0023 |
| 8 | 10 | 93.68 | ±0.37 | 0.0039 |
| 9 | 10 | 93.36 | ±0.19 | 0.0020 |
| 10 | 10 | 93.22 | ±0.11 | 0.0012 |
| 11 | 10 | 92.49** | ±0.17 | 0.0018 |
| 12 | 10 | 92.02 | ±0.14 | 0.0015 |
| 13 | 10 | 91.13 | ±0.44 | 0.0048 |
| 14 | 10 | 89.88 | ±0.22 | 0.0024 |
| 15 | 10 | 87.08 | ±0.82 | 0.0094 |
| 16 | 10 | 84.69* | ±1.49 | 0.0176 |
| 17 | 10 | 82.54 | ±0.81 | 0.0098 |
| 18 | 10 | 81.39*&** | ±0.30 | 0.0037 |
| 19 | 10 | 81.22 | ±0.87 | 0.0107 |
| 20 | 10 | 81.13 | ±1.42 | 0.0175 |

* significant difference between control and standard
** significant difference between control and experimental

TABLE XIII

MEAN WET WEIGHT (GRAMS) EXPERIMENTALS

| DAY | N= | MEAN | STD. DEV. | COEF. OF VAR. |
|---|---|---|---|---|
| 4 | 11 | 0.0681 | ±0.0108 | 0.1580 |
| 5 | 13 | 0.1909 | ±0.0352 | 0.1846 |
| 6 | 10 | 0.4489 | ±0.0640 | 0.1427 |
| 7 | 12 | 0.6888 | ±0.1454 | 0.2111 |
| 8 | 10 | 1.1718 | ±0.1376 | 0.1174 |
| 9 | 10 | 1.5728*** | ±0.0841 | 0.0535 |
| 10 | 10 | 2.1878 | ±0.1105 | 0.0505 |
| 11 | 10 | 3.1181 | ±0.3276 | 0.1051 |
| 12 | 10 | 4.5726 | ±0.5389 | 0.1179 |
| 13 | 10 | 6.5819 | ±0.5986 | 0.0909 |
| 14 | 10 | 8.2095*** | ±0.6436 | 0.0784 |
| 15 | 10 | 11.4954 | ±0.9383 | 0.0816 |
| 16 | 10 | 15.0381 | ±1.7344 | 0.1153 |
| 17 | 10 | 18.2259 | ±1.2361 | 0.0678 |
| 18 | 10 | 20.8968 | ±3.7403 | 0.1790 |
| 19 | 10 | 24.4642 | ±3.2992 | 0.1349 |
| 20 | 10 | 27.7066 | ±3.3284 | 0.1201 |

*** significant difference between control and experimental

TABLE XIV

MEAN DRY WEIGHT (GRAMS) EXPERIMENTALS

| DAY | N= | MEAN | STD. DEV. | COEF. OF VAR. |
|---|---|---|---|---|
| 4 | 11 | 0.0047*** | ±0.0011 | 0.2255 |
| 5 | 13 | 0.0108 | ±0.0017 | 0.1582 |
| 6 | 10 | 0.0255 | ±0.0032 | 0.1252 |
| 7 | 12 | 0.0403 | ±0.0093 | 0.2320 |
| 8 | 10 | 0.0728 | ±0.0083 | 0.1142 |
| 9 | 10 | 0.1049 | ±0.0068 | 0.0648 |
| 10 | 10 | 0.1483 | ±0.0115 | 0.0778 |
| 11 | 10 | 0.2264 | ±0.0220 | 0.0970 |
| 12 | 10 | 0.3582 | ±0.0593 | 0.1654 |
| 13 | 10 | 0.5748 | ±0.0592 | 0.1030 |
| 14 | 10 | 0.8340*** | ±0.0825 | 0.0989 |
| 15 | 10 | 1.4540 | ±0.1431 | 0.0984 |
| 16 | 10 | 2.3900 | ±0.4355 | 0.1822 |
| 17 | 10 | 3.1483 | ±0.3433 | 0.1090 |
| 18 | 10 | 3.6005 | ±0.9403 | 0.2612 |
| 19 | 10 | 4.6649 | ±0.5823 | 0.1248 |
| 20 | 10 | 5.1497 | ±0.9564 | 0.1857 |

*** significant difference between control and experimental

TABLE XV

MEAN ASH WEIGHT (GRAMS) EXPERIMENTALS

| DAY | N= | MEAN | STD. DEV. | COEF. OF VAR. |
|---|---|---|---|---|
| 4 | 11 | 0.0005 | ±0.0003 | 0.5357 |
| 5 | 13 | 0.0014 | ±0.0003 | 0.2052 |
| 6 | 10 | 0.0031 | ±0.0005 | 0.1506 |
| 7 | 12 | 0.0057**&*** | ±0.0014 | 0.2440 |
| 8 | 10 | 0.0093 | ±0.0016 | 0.1706 |
| 9 | 10 | 0.0137 | ±0.0011 | 0.0781 |
| 10 | 10 | 0.0219 | ±0.0014 | 0.0639 |
| 11 | 10 | 0.0313 | ±0.0039 | 0.1239 |
| 12 | 10 | 0.0523*** | ±0.0063 | 0.1214 |
| 13 | 10 | 0.0757 | ±0.0080 | 0.1051 |
| 14 | 10 | 0.1028*** | ±0.0092 | 0.0895 |
| 15 | 10 | 0.1499 | ±0.0115 | 0.0765 |
| 16 | 10 | 0.2020 | ±0.0453 | 0.2241 |
| 17 | 10 | 0.2566 | ±0.0323 | 0.1260 |
| 18 | 10 | 0.3103 | ±0.0549 | 0.1769 |
| 19 | 10 | 0.4032 | ±0.0374 | 0.0927 |
| 20 | 10 | 0.4496 | ±0.0591 | 0.1314 |

** significant difference between standard and experimental
*** significant difference between control and experimental

TABLE XVI

MEAN ORGANIC WEIGHT (GRAMS) EXPERIMENTALS

| DAY | N= | MEAN | STD. DEV. | COEF. OF VAR. |
|---|---|---|---|---|
| 4 | 11 | 0.0042*** | ±0.0010 | 0.2330 |
| 5 | 13 | 0.0094 | ±0.0015 | 0.1563 |
| 6 | 10 | 0.0224 | ±0.0031 | 0.1376 |
| 7 | 12 | 0.0346 | ±0.0081 | 0.2348 |
| 8 | 10 | 0.0635 | ±0.0082 | 0.1289 |
| 9 | 10 | 0.0912 | ±0.0060 | 0.0656 |
| 10 | 10 | 0.1264** | ±0.0102 | 0.0809 |
| 11 | 10 | 0.1951 | ±0.0304 | 0.1558 |
| 12 | 10 | 0.3059 | ±0.0534 | 0.1777 |
| 13 | 10 | 0.4991 | ±0.0523 | 0.1048 |
| 14 | 10 | 0.7312*** | ±0.0738 | 0.1010 |
| 15 | 10 | 1.3041 | ±0.1325 | 0.1016 |
| 16 | 10 | 2.1880 | ±0.3988 | 0.1823 |
| 17 | 10 | 2.8917 | ±0.3337 | 0.1154 |
| 18 | 10 | 3.2902 | ±0.8862 | 0.2694 |
| 19 | 10 | 4.2617 | ±0.5520 | 0.1295 |
| 20 | 10 | 4.7001 | ±0.9018 | 0.1919 |

** significant difference between standard and experimental
*** significant difference between control and experimental

TABLE XVII

MEAN WATER WEIGHT (GRAMS) EXPERIMENTALS

| DAY | N= | MEAN | STD. DEV. | COEF. OF VAR. |
|---|---|---|---|---|
| 4 | 11 | 0.0634 | ±0.0100 | 0.1582 |
| 5 | 13 | 0.1801 | ±0.0337 | 0.1869 |
| 6 | 10 | 0.4233 | ±0.0661 | 0.1440 |
| 7 | 12 | 0.6485 | ±0.1363 | 0.2102 |
| 8 | 10 | 1.0990 | ±0.1307 | 0.1190 |
| 9 | 10 | 1.4680*** | ±0.0782 | 0.0533 |
| 10 | 10 | 2.0395 | ±0.1047 | 0.0513 |
| 11 | 10 | 2.8917 | ±0.3077 | 0.1064 |
| 12 | 10 | 4.2144 | ±0.4820 | 0.1144 |
| 13 | 10 | 6.0071 | ±0.5454 | 0.0908 |
| 14 | 10 | 7.3755*** | ±0.5652 | 0.0766 |
| 15 | 10 | 10.0414 | ±0.8074 | 0.0804 |
| 16 | 10 | 12.6481 | ±1.3120 | 0.1037 |
| 17 | 10 | 15.0776 | ±1.0463 | 0.0694 |
| 18 | 10 | 17.2962 | ±2.8279 | 0.1633 |
| 19 | 10 | 19.7982 | ±2.7391 | 0.1383 |
| 20 | 10 | 22.5569 | ±2.4051 | 0.1066 |

*** significant difference between control and experimental

TABLE XVIII

MEAN PER CENT WATER OF WET WEIGHT EXPERIMENTALS

| DAY | N= | MEAN | STD. DEV. | COEF. OF VAR. |
|---|---|---|---|---|
| 4 | 11 | 93.13 | ±1.04 | 0.0111 |
| 5 | 13 | 94.33** | ±0.34 | 0.0036 |
| 6 | 10 | 94.29 | ±0.27 | 0.0029 |
| 7 | 12 | 94.16 | ±0.38 | 0.0040 |
| 8 | 10 | 93.76 | ±0.46 | 0.0049 |
| 9 | 10 | 93.33 | ±0.22 | 0.0024 |
| 10 | 10 | 93.22 | ±0.44 | 0.0047 |
| 11 | 10 | 92.73** | ±0.29 | 0.0031 |
| 12 | 10 | 92.21 | ±0.55 | 0.0060 |
| 13 | 10 | 91.27 | ±0.40 | 0.0044 |
| 14 | 10 | 89.85 | ±0.34 | 0.0038 |
| 15 | 10 | 87.36*** | ±0.51 | 0.0058 |
| 16 | 10 | 84.21 | ±1.22 | 0.0145 |
| 17 | 10 | 82.73 | ±1.42 | 0.0171 |
| 18 | 10 | 83.01** | ±1.74 | 0.0209 |
| 19 | 10 | 80.89 | ±0.64 | 0.0079 |
| 20 | 10 | 81.57 | ±1.61 | 0.0197 |

** significant difference between standard and experimental
*** significant difference between control and experimental

TABLE XIX

GROWTH RATE EQUATION
$\log y = \log aa + (bb \log c)x$

| | GROUP | Y-INTERCEPT | SLOPE | GROWTH RATE |
|---|---|---|---|---|
| WET | Cont. | 0.0506 | 0.3504 | 0.4196 |
| | Std. | 0.0531 | 0.3455 | 0.4127 |
| | Exp. | 0.0516 | 0.3457 | 0.4129 |
| DRY | Cont. | 0.0017 | 0.4393 | 0.5524 |
| | Std. | 0.0018 | 0.4323 | 0.5408 |
| | Exp. | 0.0017 | 0.4319 | 0.5401 |
| ASH | Cont. | 0.0003 | 0.4034 | 0.4969 |
| | Std. | 0.0003 | 0.3904 | 0.4776 |
| | Exp. | 0.0003 | 0.4008 | 0.4930 |
| ORGANIC | Cont. | 0.0014 | 0.4437 | 0.5585 |
| | Std. | 0.0015 | 0.4374 | 0.5487 |
| | Exp. | 0.0014 | 0.4358 | 0.5462 |
| WATER CONTENT | Cont. | 0.0515 | 0.3393 | 0.4040 |
| | Std. | 0.0539 | 0.3347 | 0.3976 |
| | Exp. | 0.0524 | 0.3351 | 0.3981 |
| % WATER CONTENT | Cont. | 101.7 | -0.0110 | -0.0110 |
| | Std. | 101.5 | -0.0107 | -0.0107 |
| | Exp. | 101.3 | -0.0105 | -0.0104 |